2022

Impact of Dry, Particle-Size Fractionation On Protein And Amino Acid Content Of Three Seaweed Species

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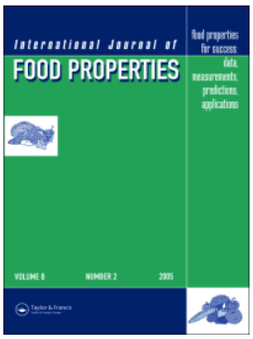
Recommended Citation

Healy, L.E., Zhu, X. & Pojic, M. (2022). Impact of dry, particle-size fractionation on protein and amino acid content of three seaweed species. *International Journal of Food Properties*, vol. 25, no. 1, pg. 2073-2088. doi:10.1080/10942912.2022.2120001

Funder: Teagasc Walsh Scholarship and the BiOrbic SFI Bioeconomy Research Centre, which is funded by Ireland's European Structural and Investment Programmes, Science Foundation Ireland (16/ RC/3889) and the European Regional Development Fund



International Journal of Food Properties

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ljfp20

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To cite this article: Laura E Healy, Xianglu Zhu, Milica Pojic, Mahesha M Poojary, James Curtin, Uma Tiwari, Carl Sullivan & Brijesh K Tiwari (2022) Impact of dry, particle-size fractionation on protein and amino acid content of three seaweed species, International Journal of Food Properties, 25:1, 2073-2088, DOI: 10.1080/10942912.2022.2120001

To link to this article: https://doi.org/10.1080/10942912.2022.2120001

INTERNATIONAL JOURNAL OF FOOD PROPERTIES
2022, VOL. 25, NO. 1, 2073–2088
https://doi.org/10.1080/10942912.2022.2120001

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Impact of dry, particle-size fractionation on protein and amino acid content of three seaweed species

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ABSTRACT

Market demand for "clean and green" food products is increasing, and so there is growing opportunity for the seaweed aquaculture industry to take a position as a key food producer in this area. In this study, in order to investigate the impact of dry fractionation on seaweed protein qualities, dried and milled seaweed powder from three seaweed species was sieved into 6 fractions (F1 to F6) of different particle size from >710 µm to <50 µm. True protein, total protein and amino acid profiles were analyzed to evaluate the protein content and quality of three brown seaweed species commercially harvested in Ireland; *Alaria esculenta, Laminaria digitata* and *Saccharina latissima*. In general, *A. esculenta* had the highest protein content, followed by *S. latissima* and then *L. digitata* (4.15 ± 0.12 g/100 g, 2.28 ± 0.1 g/100 g and 1.73 ± 0.01 g/100 g, respectively). Fractionation had a significant impact ($p < .01$) on protein content, essential amino acid content ($p < .05$) and non-essential amino acid content ($p < .01$) across six fractions of seaweed powder within species. F6 (<50 was the fraction that contained the highest protein and amino acid content in both *A. esculenta* and *S. latissima*. F1 (>710 µm) contained the highest protein and amino acid content in *L. digitata*. Glutamic acid was the most prevalent amino acid in *A. esculenta* and *L. digitata* (55.34 mg/g and 23.78 mg/g), while aspartic acid was the most prevalent in *S. latissima* (19.41 mg/g). This information is valuable to both researchers and seaweed producers who can use particle size separation as a simple method to create value-added products using their green biomass for applications across multiple markets.

ARTICLE HISTORY
Received 13 July 2022
Revised 24 August 2022
Accepted 28 August 2022

KEYWORDS
Seaweed; Particle size; fractionation; True protein; Total protein; Amino acids

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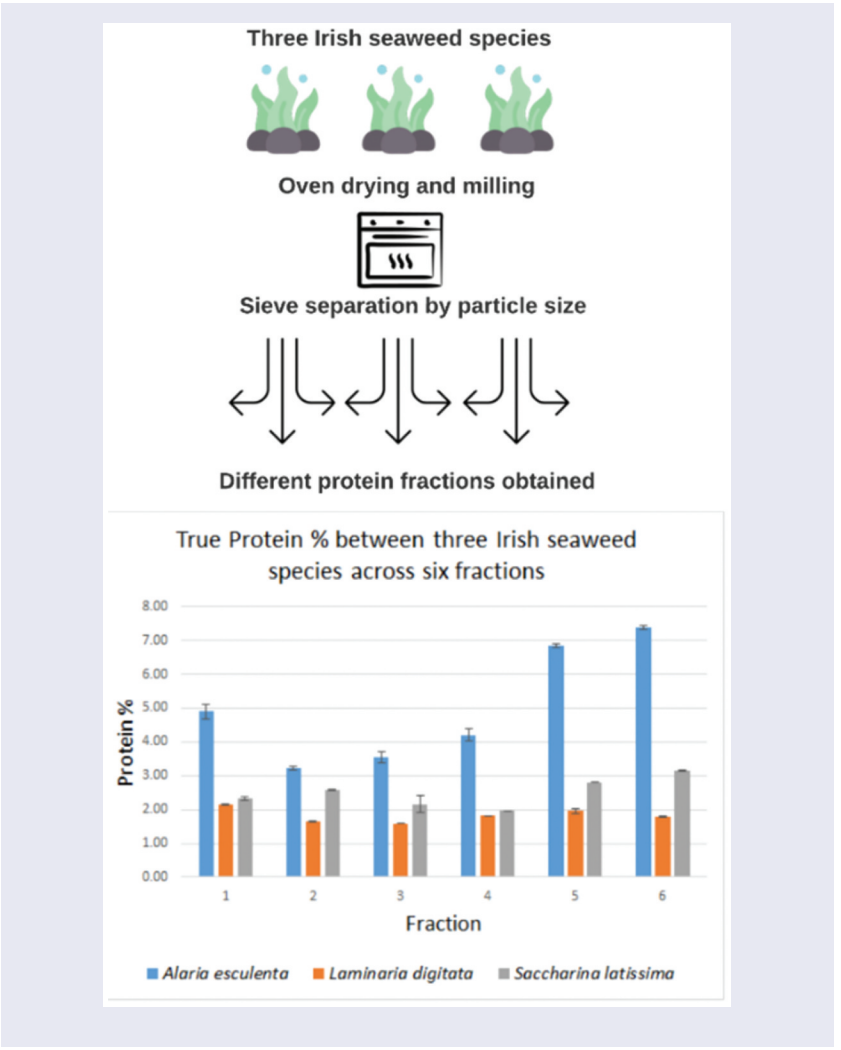

Introduction

In Ireland, seaweed grows in both great abundance and diversity, where over 570 species can be found .[1] *A. esculenta* (also known as winged kelp), *L. digitata* (also known as oarweed) and *S. latissima* (also known as sugar kelp) are three species of brown seaweed commercially harvested in Ireland. This paper aimed to investigate the impact of fractionation on the protein and amino acid profile of these three seaweed species. *A. esculenta* and *S. latissima* are considered popular seaweed species for use in food products due to the potential health benefits offered (such as antioxidant activity) .[2] Brown seaweed species can also turn green when heated, making them more attractive to the consumer .[2] *L. digitata*, however, is a lesser used brown kelp species but with potential applications being discovered, the species has gained more interest in a range of commercial industries such as cosmeceuticals, functional foods and bioplastics .[3] All three species are grown in Ireland and were accessible for the purpose of this research. With this in mind, these three species were selected. Seaweed can be a rich source of protein .[4] Some studies show that green and red seaweeds contain more protein (10–47% DW) than brown (5–24% DW) .[4,5] Variation can also occur within species, depending on season, environmental conditions and time of harvest,[5] as is seen in *Palmaria palmata*,[6] *L. digitata* and *Ulva lactuca* .[7] The amino acid profile of any given food product is a reference for the quality

of the protein content. Seaweed contains all essential amino acids required for human nutrition .[8] Seaweeds are also known for their high antioxidant content (such as fucoxanthin) which makes them ideal candidates for inclusion into food as an ingredient to offer health benefits to the consumer[9] that could help in the prevention of chronic diseases such as obesity, cardiovascular disease and diabetes .[10,11]

New product development

Seaweed can be incorporated into a range of products such as muffins,[12] noodles,[13] cakes,[14] jelly, soup and ice-cream to add-value .[15] In a study by Mamat *et al.* (2014) on the use of a red seaweed *Kappaphycus alvarezii* to enhance bread textural properties, the addition of this seaweed powder to dough (at a rate of 2–8%) increased water absorption of the dough, decreased stickiness and showed improved firmness .[16] Beverages such as coffee infused with seaweed powder was found to have more ferric reducing antioxidant power .[17] An instant seaweed powder drink was developed as a healthy alternative to common fruit-based beverages as a means of delivering fiber to the consumer .[18] The end-product destination influences the manufacturer's processing strategy. For instance, for baked goods, seaweed will need to be milled and sieved down to the correct fraction size for the desired flour grade.

Fractionation is a non-thermal, nondestructive and low-energy consuming process of separating biomass (in this case seaweed powder) into fractions based on particle size distribution. Flours are distinguished by their particle size for different applications in cooking. The differences in crumb size of the flour affects how much water is needed in the baking process and has an impact on texture of the final product .[19] For instance, 00 flour is a finely ground wheat flour used in pizza making with a particle size of 71.686 μm,[20] while semolina refers to a type of wheat flour that contains coarser flour, where no more than 10% of the material passes through a 180 μm sieve .[21] The type of fractionation used in this study can be referred to as "dry" due to it being carried out on dried biomass as opposed to wet biomass.

Two protein methods are compared; "True Protein" and "Dumas combustion" method, for each fraction in each species in order to detect any discrepancies between testing methods. "True Protein" is a measure of protein content of a substrate based on the total amino acids contained. "Dumas combustion" is a method of determining nitrogen content of a substrate based on sample combustion at high temperatures in an oxygenated atmosphere. The resulting figure is then converted to a protein % (g/100 g) based on a standard nitrogen factor which varies for different food types. The results discussed in this study have implications for researchers, innovators and seaweed producers alike, who may have an interest in determining the most effective way of using seaweed biomass as a novel protein source.

Materials and methods

Sampling of seaweed and pre-treatment

Three seaweed species were analyzed for this study; *A. esculenta, L. digitata* and *S. latissima*. All seaweed samples were collected in March 2020 from Dúlra, an organic seaweed aquaculture farm in Co. Mayo, Ireland. The samples were identified by a specialist at the farm prior to packaging for analysis. The seaweed samples underwent the following post-harvest treatments: (i) Washing in a large container, with tap water to remove any debris such as sand. (ii) Drying using a HiDew dehumidifier (SP075007) in a sealed and insulated room with air circulation for moisture removal. Drying temperature was maintained at an average of 38–40°C. (iii) Milling using an Alvan Blanch stainless steel hammermill with a 10 mm screen. The seaweed powder had a moisture content of about 10% after these post-harvest steps and was measured using an Infrared Moisture Analyzer MA35.

Fractionation of seaweed

Milled seaweed flour was fractionated for analysis. Fractionation was carried out by an EML 200 premium automatic sieve shaker (VWR, Avantor, PA, USA). A sample of 500 g of whole seaweed flour of each species was passed through the automatic sieve shaker with the following sieve component trays in place: >710 μm, >500 μm, >250 μm, >100 μm, >50 μm and <50 μm. This meant that a total of six fractions (F1 – F6) of seaweed flour would be collected for analysis. The weight of each resulting fraction was recorded. F1 equates to all seaweed retained in the mesh size >710 μm, F2 refers to all seaweed retained in the mesh greater than 500 μm but less than 710 μm, and so on.

Protein analysis

Protein concentration of all samples was determined using two methods for purposes of comparison. The first was "True Protein" which includes protein, free amino acids and peptides. It was calculated as follows: True protein (% w/w sample, or g/ 100 g sample) = total amino acids (g/ 100 g sample) x (100/116) .[22] The second method to calculate protein concentration was determined using a LECO FP628 (LECO Corp., MI, USA) protein analyzer based on the Dumas method and according to AOAC method 992.15, 1990. The nitrogen to protein conversion used was 6.25. The equation used was as follows: Total protein = total nitrogen x 6.25. This method is referred to as the "Dumas combustion" method.

Amino acid analysis

Amino acid analysis was carried out as follows: Initially, the samples were hydrolyzed at 110°C for 23 h using 4 M methanesulfonic acid containing 0.2% w/v tryptamine. The hydrolyzates were neutralized with equal volumes of 4 M NaOH. The diluted samples were subjected to amino acid analysis using a protocol from Hildebrand et al., 2020 .[23] A portion (200 μL) of each seaweed sample was mixed with equal volume of an internal standard (50 μM of 6-aminocaproic acid) and filtered through 0.22 μm regenerated cellulose membrane filters. Samples were then derivatized with o-phthalaldehyde (for primary amino acids) and 9-fluorenylmethoxycarbonyl chloride (for secondary amino acids) before their injection into a UHPLC-FLD system (Thermo Ultimate 3000 RS, Thermo Scientific, USA) equipped with an Agilent AdvanceBio AAA column (100 mm × 3.0 mm ID × 2.7 μm particle size, Agilent Technologies, USA). The separation was performed using two mobile phases; mobile phase A (10 mM Na_2HPO_4 in 10 mM $Na_2B_4O_7$ decahydrate, pH 8.2) and mobile phase B (mixtures 45:45:10, v:v:v of acetonitrile, methanol and water) at a flow rate of 0.62 mL min^{-1} following a gradient program. The fluorescence detection was carried out at wavelengths of 340 nm (excitation) and 450 nm (emission). The results are expressed as mg of amino acid per g of fractionated sample.

Data analysis

Statistical analysis was carried out on R (Version 1.1.463 – © 2009–2018 RStudio, Inc.). The differences in the measurement of protein by two different methods, the effect of fractionation on protein content and amino acid profile as well as the differences in these profiles across species were analyzed by one-way ANOVA analyses. A Tukey's Post-Hoc test was also carried out to determine the levels of significance in protein content between six fractions, within each species. In all cases, the criterion for statistical significance was $p \leq .05$.

Results and discussion

Distribution of biomass across fractions

When sieved, different proportions of seaweed were found in each fraction (F1 – F6). The breakdown of this is summarized in Figure 1. In all three species the highest proportion of

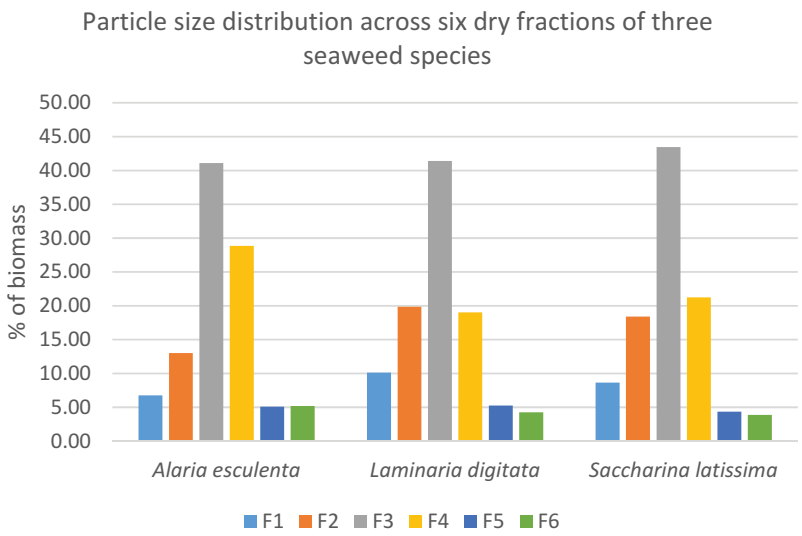

Figure 1. Particle size distribution across six fractions of seaweed (three different species). (Where F1 = >710 µm, F2 = 500–710 µm, F3 = 250–500 µm, F4 = 100–250 µm, F5 = 50–100 µm, F6 = <50 µm).

biomass was found in fraction three (F3). In *A. esculenta*, 41.09% of the biomass was found in F3 while accounting for 41.4% and 43.45% in *L. digitata* and *S. latissima* respectively. For *L. digitata* and *S. latissima*, F6 held the smallest quantity of seaweed powder (accounting for 4.27% and 3.87%, respectively), while F5 contained the least amount of powder (5.09%) for *A. esculenta.*

Protein content across three Irish seaweed species

Protein was examined by two methods; "True Protein" method and "Dumas combustion" method. The type of protein analysis method used yielded significantly different results ($p < .01$) across species. Table 1 shows the breakdown of protein content by these two protein analysis methods, across all six fractions and three seaweed species. Although the total protein results vary significantly between methods, the highest and lowest protein content for each fraction are consistent across methods for *A. esculenta*, where F6 is determined to be the fraction containing the highest protein content at 7.40 ± 0.05 g/100 g for "True protein" method and 12.83 ± 0.10 g/100 g for "Dumas combustion" method. F2 was found to be the fraction containing the least amount of protein in *A. esculenta* powder, across the two methods (3.23 ± 0.04 g/100 g for "True protein" method and 7.06 ± 0.12 g/100 g for "Dumas combustion" method). In *S. latissima*, F6 also gave the highest protein figure of the two methods (3.15 ± 0.03 g/100 g for "True Protein" method and 5.70 ± 0.52 g/100 g for "Dumas combustion" protein method. The lowest protein content was found in F4 for "True Protein" (1.96 ± 0.01 g/100 g, while F3 contained the lowest amount of protein (4.15 ± 0.18 g/100 g) for "Dumas combustion" protein method in *S. latissima* (see Table 2).

Table 1. Summary of two protein determination methods for three Irish seaweed species, total amino acid %, essential amino acid content and non-essential amino acid content.

Species/ Result	Protein % "True Protein" method	Protein % "Dumas combustion" method	Total AA %	EAA (mg/g)	NEAA (mg/g)
A. esculenta	4.154 ± 0.12	7.844 ± 0.02	4.818 ± 0.14	15.780 ± 0.58	32.404 ± 0.82
L. digitata	1.729 ± 0.01	3.015 ± 0.01	2.006 ± 0.01	7.846 ± 0.06	12.210 ± 0.03
S. latissima	2.276 ± 0.10	4.430 ± 0.15	2.641 ± 0.12	9.899 ± 0.6	16.507 ± 0.57

Table 2. Protein concentration determined from two methods of protein analysis ("True Protein" and "Dumas combustion") across three seaweed species. (Where F1 = >710 µm, F2 = 500–710 µm, F3 = 250–500 µm, F4 = 100–250 µm, F5 = 50–100 µm, F6 = <50 µm).

Species	A. esculenta		L. digitata		S. latissima	
Fraction / Method	"True Protein"	"Dumas combustion"	"True Protein"	"Dumas combustion"	"True Protein"	"Dumas combustion"
F1	4.903 ± 0.210	7.955 ± 0.014	2.150 ± 0.010	3.194 ± 0.008	2.332 ± 0.057	2.705 ± 0.066
F2	3.228 ± 0.045	7.0633 ± 0.124	1.652 ± 0.018	3.166 ± 0.022	2.568 ± 0.021	2.978 ± 0.024
F3	3.551 ± 0.174	7.1046 ± 0.062	1.586 ± 0.003	3.007 ± 0.026	2.164 ± 0.254	2.510 ± 0.295
F4	4.201 ± 0.178	7.7233 ± 0.004	1.812 ± 0.004	2.609 ± 0.021	1.963 ± 0.006	2.277 ± 0.007
F5	6.836 ± 0.057	11.282 ± 0.115	1.952 ± 0.075	3.017 ± 0.061	2.804 ± 0.004	3.253 ± 0.005
F6	7.39 ± 0.051	12.827 ± 0.99	1.789 ± 0.019	3.762 ± 0.223	3.153 ± 0.025	3.658 ± 0.029

Contrastingly, in *L. digitata*, F1 had the highest protein content for "True Protein" method (2.15 ± 0.01 g/100 g), while F6 had the highest protein content for "Dumas combustion" method (3.76 ± 0.22 g/100 g). It is not exactly clear why the protein is distributed unevenly across the different fractions across the different species. It is known that the seaweed cell wall complex is made up of crystalline cellulose microfibrils which function as structural agents for the plant,[24,25] with species-specific groups of functional polysaccharides such as alginate or fucoidan immersed in this matrix. The species-specific differences by which these components make up the seaweed tissue on the cellular level may offer insight into the protein distribution differences observed in this study. For instance, the protein molecules may be more tightly bound in *L. digitata*, and therefore not as easily released from the cellular matrix upon milling, as was contrastingly observed in the other two species. A follow up study should look at SEM photography for observable cell-structural differences post-fractionation. A follow up study testing digestibility of seaweed fractions such as those produced by this study may reveal the significance, if any, of the separation of these proteins from the other seaweed components on human digestibility.

"True Protein" figures were selected for comparison of protein content between the three examined species using a one-way ANOVA. The reason for selecting this method over "Dumas combustion" method was the lack of clarity over which nitrogen factor to use for the specific seaweed species used in this study .[26] There was a significant difference in protein content ($p < .05$) between the three Irish seaweed species studied (Figure 2). *A. esculenta* has the highest protein content out of the three species studied, followed by *S. latissima* and *L. digitata* (Table 2). Table 6 shows the levels of significance of protein content between fractions within the three seaweed species.

The results of this study show that, depending on the test used to analyze protein content, significantly different results can be obtained. This has a major implications for the seaweed producer, who needs to be able to assure their customers of consistent nutritional information. Companies may choose to test their products in a manner that yields the most desirable results (in this case a high protein content) as long as all methods of analysis are approved. Clearer guidelines on the analysis of protein for seaweed for human consumption should be further explored, with recommendations for a standard method proposed. Angell, A.R. *et al.* studied the different seaweed protein quantification methods employed in research and found that 42% of all studies applied direct extraction procedures (such as employing the use of assays), while 52% applied an indirect nitrogen-to-protein conversion factor or 6.25 .[27] Discussing the various methods of protein analysis in seaweed, they highlight the inconsistencies and potential inaccuracies in protein content reporting .[27] They propose a nitrogen factor of 5 be used when analyzing seaweed protein content to replace the standard 6.25 factor due to results from their meta-analysis concluding that a factor of 6.25 leads to an overestimation of protein by 43% .[27] Contrastingly, Mæhre, H.K. *et al.*, (2018) propose that calculation of protein content based on amino acid content was the most reliable method, although they state that the hydrolysis method involved could be improved .[28]

To investigate the effect of fractionation on protein content *within species*, a one-way ANOVA was carried out on fractionated seaweed from each species separately. All results showed a significant difference ($p < .01$), meaning fractionation had an impact on the protein

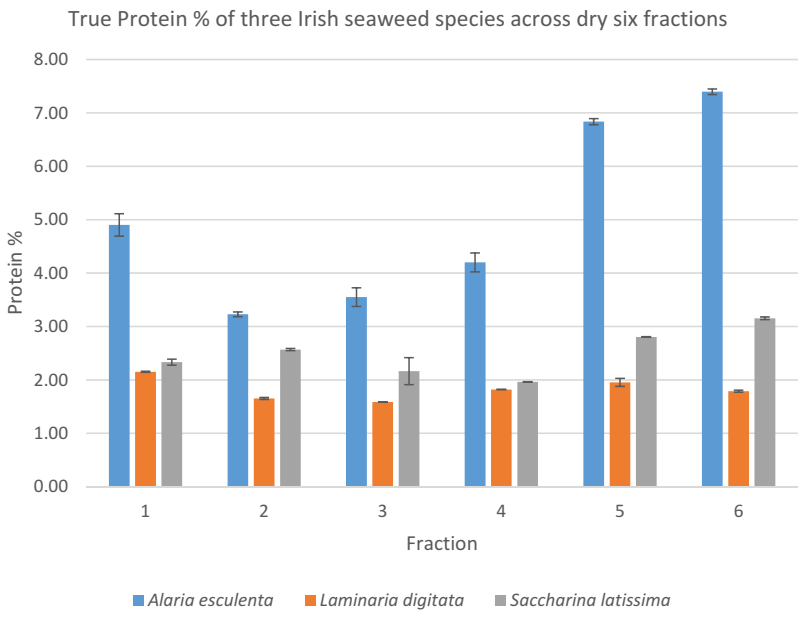

Figure 2. "True Protein" % between three Irish seaweed species across six fractions. (Where F1 = >710 µm, F2 = 500–710 µm, F3 = 250–500 µm, F4 = 100–250 µm, F5 = 50–100 µm, F6 = <50 µm).

content of the seaweed between fractions within individual seaweed species. Figure 2 is a bar chart displaying the protein content of each seaweed across six fractions of different particle sizes.

Differences in protein content across fractions of different particle sizes has been widely reported in wheat, but never in seaweed, as far as the authors of this study could find. Two species of wheat (hard red winter wheat and hard white winter wheat) were fractionated by sieving to see the impact of the process on textural properties of tortillas made with the fractioned flours .[29] The medium flours (38–53 µm and 53–75 µm) had a higher protein content than the finest and coarsest fractions (< 38 µm and > 75 µm) .[29] The finest flour fraction (< 38 µm) was associated with a poorer rupture distance and foldability, possibly due to the higher concentration of lower molecular weight proteins and high amount of damaged starch .[29] Ma, S. *et al.* (2019) found that particle size of wheat could be a predictor of product quality as they observed a major effect on quality attributes such as cooking and textural properties as well as negative impacts on overall quality .[30]

Amino acid profile of three Irish seaweed species

Overall, 16 amino acids were detected in the three seaweed species examined. All essential amino acids were present in the three species, with the exception of tryptophan, which was not detected. Various amino acid extraction methods have different drawbacks, one common outcome of protein hydrolysis is that certain amino acids like tryptophan or cysteine can be destroyed in the process[31] leading to a slight under-estimation of the total protein content of the sample, if deriving from amino acid content. All three seaweed species had the same top 5 amino acids in terms of mg/g. *A. esculenta* and *L. digitata* had the same amino acids in the same order of content, with glutamic acid being the dominant amino acid of the two species, while aspartic acid was the dominant amino acid in *S. latissima* (Table 3). Leucine was the only essential amino acid to make it into the top 5 of all amino acids in the three species. All detected amino acids were spread across all fractions of seaweed species examined (see full raw data in Table 5).

Table 3. The top five by proportion (mg/g DW seaweed) amino acids found in each of the three seaweed species.

Species	Glutamic acid	Aspartic acid	Glycine	Alanine	Leucine
A. esculenta	55.338	40.484	36.219	35.636	24.693
L. digitata	23.776	15.424	14.544	13.419	12.776
S. latissima	18.397	19.406	10.589	9.997	12.549

As observed in other studies, glutamic acid is a common feature in the amino acid profiles of seaweed, particularly brown seaweed .[32] This amino acid is responsible for the "umami" taste in seaweed when present in its free amino acid form and because of this; seaweeds can be used as a source of obtaining this flavor for foods. These findings correlate with other studies that found aspartic acid and glutamic acid to be the most concentrated amino acids across a range of 34 products that contained 5 different seaweed species .[33] It was also found that brown algae contained significantly higher amounts of these amino acids when compared to red algae .[33] Tryptophan was the first limiting amino acid also found for all species studied by Dawczynski. C. *et al.* (2007), with leucine and isoleucine found to be limited in red species while methionine, cystine and lysine found to be limited in brown species. In this study, histidine was consistently low in all three species examined (4.65 mg/g in *A. esculenta*, 1.25 mg/g in *L. digitata* and 0.69 mg/g in *S. latissima*), followed next by methionine (5.75 mg/g in *A. esculenta*, 2.77 mg/g in *L. digitata* and 2.04 mg/g in *S. latissima*). This information is of importance in the context of human and animal nutrition as methionine can be deficient in diets.

Essential and non-essential amino acid content across three Irish seaweed species

Total essential amino acid content was significantly different ($p < .01$) between the three seaweed species examined. *A. esculenta* had the highest essential amino acid (EAA) content of all three species examined at 15.78 ± 0.58 mg/g, followed by *S. latissima* at 9.9 ± 0.6 mg/g. *L. digitata* had the lowest EAA content at 7.85 ± 0.06 mg/g (Table 1).

Total non-essential amino acid content was significantly different ($p < .01$) between the three seaweed species examined. *A. esculenta* had the highest non-essential amino acid (NEAA) content of all three species examined at 32.4 ± 0.82 mg/g. *S. latissima* had the next highest NEAA content at 16.51 ± 0.57 mg/g, with *L. digitata* having the lowest NEAA content at 12.21 ± 0.03 mg/g (Table 1). Figure 3 shows the differences in total amino acid content (both essential and non-essential) between species.

When examining the EAA content of red and brown seaweed groups, Dawczynski. C. *et al.*, (2007) found that red seaweed species had similar EAA concentrations, while brown seaweed species differed significantly,[33,34] a finding that correlates with the results of this study. Studying a range of red, green and brown seaweed species it was determined that all EAA were found in sufficient amounts, except for methionine and semiessential cysteine .[35] However, even as a limiting amino acid, methionine was still found to be three times higher in these seaweeds than in soy and so the authors propose supplementation of fish meal with seaweed could help meet nutritional standards in the development of fish feed for fish farming .[35]

When examining the impact of fractionation on essential amino acid (EAA) content within species, the results were significant for *A. esculenta* ($p < .01$), *L. digitata* ($p < .01$) and *S. latissima* ($p < .05$) (Table 4). Figures 4, 5 and 6 show the breakdown of EAA and NEAA across six fractions of *A. esculenta, L. digitata* and *S. latissima* respectively. Fractionation was found to have a significant impact on non-essential amino acid (NEAA) content within species; *A. esculenta* ($p < .01$), *L. digitata* ($p < .01$) and *S. latissima* ($p < .01$). The relationship between protein content and amino acid content can clearly be observed in Figures 7, 8 and 9, which is expected since the protein content was calculated based on amino acid content. It can be seen that the highest EAA and NEAA contents are found in F1 of *L. digitata*, corresponding with the higher total protein content found in this fraction for this species.

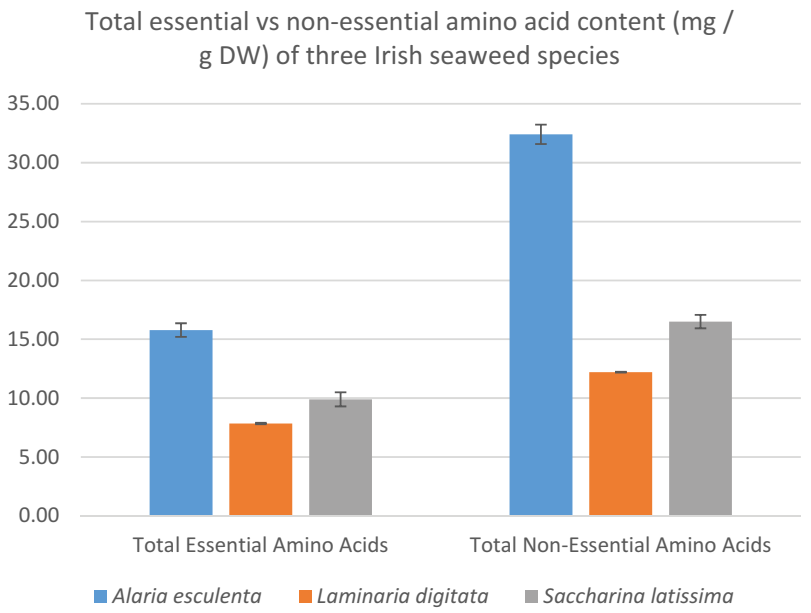

Figure 3. Total essential vs non-essential amino acid content (mg/ g DW) of three Irish seaweed species.

Table 4. Summary of EAA and NEAA content (mg/g DW seaweed) of dried powder of three Irish seaweed species across six fractions of different particle sizes.

Species	Total AA content (mg/g)	F1	F2	F3	F4	F5	F6
A. esculenta	EAA	19.386 ± 0.24	12.950 ± 0.06	13.839 ± 0.83	15.405 ± 0.92	23.989 ± 0.12	27.615 ± 0.38
	NEAA	37.490 ± 2.2	24.503 ± 0.46	27.340 ± 1.19	33.326 ± 1.14	55.303 ± 0.79	58.182 ± 0.98
L. digitata	EAA	9.735 ± 0.22	7.433 ± 0.07	7.138 ± 0.06	8.364 ± 0.00	9.333 ± 0.12	8.010 ± 0.18
	NEAA	15.209 ± 0.34	11.735 ± 0.14	11.261 ± 0.03	12.745 ± 0.05	13.315 ± 0.75	12.741 ± 0.04
S. latissima	EAA	10.207 ± 0.12	11.377 ± 0.10	9.251 ± 1.39	8.769 ± 0.02	11.848 ± 0.35	13.467 ± 0.23
	NEAA	16.846 ± 0.54	18.407 ± 0.14	15.853 ± 1.55	14.002 ± 0.04	20.681 ± 0.40	23.111 ± 0.52

Relevance to industry

The plant cell wall is a characteristic feature of plant cells which is made up of "biopolymers such as polysaccharides, phenolic compounds and various proteins" which contribute to the plants mechanical strength and rigidity .[36] In seaweed, polysaccharides and proteins are linked within the cell matrix. This makes the solubilization of seaweed protein challenging using typical extraction methods due to the possibility of denaturation of the protein fraction .[37] Different studies have examined different methods to extract proteins from seaweed, such as enzymatic and chemical extraction, in an attempt to break these links between polysaccharides and protein .[37–43]

The interaction between seaweed proteins and polysaccharides are not well documented, and in particular how these interactions impact the techno-functional properties of food products developed with seaweed. Based on this and the evidence collected by this study, further examination into the distribution of seaweed polysaccharides across fractions, as well as the interactions of protein and these polysaccharides and their impact on end-product quality is recommended. For those seaweed producers looking for a simple way to separate different fractions of their seaweed to produce products with different applications based on

Table 5. Amino Acid profiles of three Irish seaweeds across six different fractions of powder. Units are mg of amino acid per g of DW seaweed powder.

		Amino Acid (mg/g)																	
Species	Fraction	Asp mg/g	Glu mg/g	Ser mg/g	His mg/g	Gly mg/g	Thr mg/g	Arg mg/g	Ala mg/g	Tyr mg/g	Val mg/g	Met mg/g	Trp mg/g	Phe mg/g	Ile mg/g	Leu mg/g	Lys mg/g	Pro mg/g	Hyp mg/g
Alaria esculenta	F1	6.707	9.146	3.790	0.721	6.024	2.262	2.998	5.890	2.183	2.675	1.109	ND	2.775	1.773	4.316	3.990	2.951	ND
	F1	6.447	8.027	3.466	0.782	4.463	2.751	2.662	5.525	2.172	2.627	0.943	ND	2.638	1.679	4.145	3.585	2.529	ND
	F2	4.333	5.394	2.365	0.488	3.215	1.538	1.797	3.853	1.448	1.905	0.696	ND	1.816	1.134	2.882	2.432	1.639	ND
	F2	4.423	5.496	2.434	0.555	3.349	1.593	1.989	4.042	1.480	1.768	0.578	ND	1.851	1.149	2.969	2.546	1.749	ND
	F3	4.830	6.205	2.581	0.371	3.805	1.567	1.965	4.288	1.537	1.827	0.504	ND	1.843	1.190	3.098	2.606	0.939	ND
	F3	5.115	6.563	2.798	0.484	3.929	1.789	2.084	4.438	1.678	1.996	0.606	ND	2.112	1.316	3.413	2.956	1.923	ND
	F4	5.092	6.947	2.891	0.511	5.008	1.655	2.272	4.858	1.543	2.098	0.727	ND	1.977	1.270	3.446	2.797	3.578	ND
	F4	5.876	7.944	3.260	0.606	5.094	1.956	2.672	5.300	1.850	2.308	0.810	ND	2.220	1.447	3.724	3.260	2.467	ND
	F5	9.071	13.204	4.986	1.062	9.227	2.791	4.423	8.154	2.752	3.361	1.406	ND	3.608	2.222	5.083	4.578	2.700	ND
	F5	8.837	13.115	5.062	1.177	9.457	2.800	4.563	8.190	2.803	3.055	1.164	ND	3.497	2.247	5.151	4.773	4.065	ND
	F6	10.248	14.401	5.196	1.178	9.206	3.295	5.056	8.253	3.159	4.267	1.526	ND	3.747	2.703	5.630	5.649	1.687	ND
	F6	9.990	14.232	5.515	1.370	9.659	3.279	5.266	8.480	3.276	3.430	1.463	ND	3.984	2.629	5.529	5.550	2.739	ND
Laminaria digitata	F1	3.076	3.667	1.640	0.139	2.084	1.415	1.184	1.979	0.903	1.400	0.351	ND	1.401	0.810	2.048	1.945	1.015	ND
	F1	2.886	3.485	1.572	0.183	2.039	1.382	1.157	1.926	0.883	1.587	0.620	ND	1.376	0.869	2.001	1.941	0.922	ND
	F2	2.342	2.739	1.194	0.042	1.560	1.034	0.931	1.504	0.621	1.141	0.296	ND	1.049	0.602	1.833	1.369	0.703	ND
	F2	2.354	2.791	1.256	0.117	1.639	1.080	0.974	1.552	0.671	1.149	0.322	ND	1.014	0.581	1.787	1.451	0.638	ND
	F3	2.257	2.646	1.215	0.056	1.598	1.057	0.905	1.480	0.605	1.192	0.299	ND	0.957	0.532	1.569	1.412	0.586	ND
	F3	2.278	2.662	1.218	0.058	1.587	1.022	0.855	1.476	0.634	1.189	0.302	ND	1.083	0.576	1.546	1.425	0.518	ND
	F4	2.542	2.923	1.349	0.176	1.734	1.196	1.028	1.629	0.688	1.212	0.311	ND	1.233	0.691	2.032	1.514	0.801	ND
	F4	2.546	2.982	1.325	0.110	1.723	1.116	1.048	1.641	0.715	1.271	0.376	ND	1.246	0.709	2.041	1.494	0.815	ND
	F5	2.684	3.238	1.399	0.035	1.826	1.113	1.041	1.700	0.677	1.528	0.302	ND	1.328	0.788	2.318	1.796	ND	ND
	F5	2.754	3.335	1.484	0.104	1.946	1.211	1.147	1.822	0.743	1.335	0.359	ND	1.445	0.786	2.386	1.832	0.835	ND
	F6	2.553	3.062	1.345	0.174	1.707	1.124	1.033	1.632	0.713	1.190	0.390	ND	1.201	0.662	2.021	1.432	0.735	ND
	F6	2.574	3.053	1.298	0.125	1.716	1.093	1.019	1.628	0.672	1.132	0.314	ND	1.177	0.646	1.956	1.384	0.742	ND
Saccharina latissima	F1	3.252	3.979	1.744	0.321	2.336	1.587	1.431	2.359	1.010	1.473	0.493	ND	1.466	0.882	2.166	1.938	1.276	ND
	F1	2.977	3.739	1.679	0.288	2.315	1.469	1.230	2.204	0.931	1.521	0.555	ND	1.396	0.839	2.130	1.889	1.228	ND
	F2	3.443	4.306	1.898	0.346	2.439	1.601	1.438	2.479	1.112	1.654	0.613	ND	1.604	0.997	2.438	2.228	1.432	ND
	F2	3.342	4.153	1.846	0.361	2.471	1.609	1.554	2.518	1.102	1.653	0.646	ND	1.574	0.936	2.426	2.068	1.281	ND
	F3	2.514	3.124	1.381	0.138	2.163	0.856	1.047	1.840	0.711	1.305	0.418	ND	1.070	0.631	1.852	1.588	1.518	ND
	F3	3.313	4.056	1.797	0.286	2.319	1.632	1.488	2.441	1.009	1.507	0.448	ND	1.335	0.884	2.324	2.228	0.982	ND
	F4	2.452	3.201	1.409	0.177	2.080	1.165	1.264	1.917	0.799	1.399	0.481	ND	1.192	0.686	1.874	1.773	0.834	ND
	F4	2.483	3.227	1.403	0.177	2.083	1.153	1.300	1.971	0.778	1.394	0.478	ND	1.204	0.673	1.913	1.799	0.801	ND
	F5	3.688	4.526	1.962	0.283	3.151	1.273	1.900	2.627	1.054	1.600	0.480	ND	1.572	0.960	2.920	2.413	2.172	ND
	F5	3.390	4.411	1.932	0.333	3.079	1.322	2.159	2.634	1.110	1.751	0.678	ND	1.714	0.994	2.924	2.479	1.568	ND
	F6	4.166	4.990	2.213	0.308	3.333	1.474	2.143	2.861	1.220	1.916	0.756	ND	1.762	1.136	3.263	2.627	2.702	ND
	F6	3.791	4.907	2.125	0.428	3.361	1.506	2.419	2.909	1.254	1.931	0.810	ND	1.878	1.136	3.251	2.754	1.830	ND

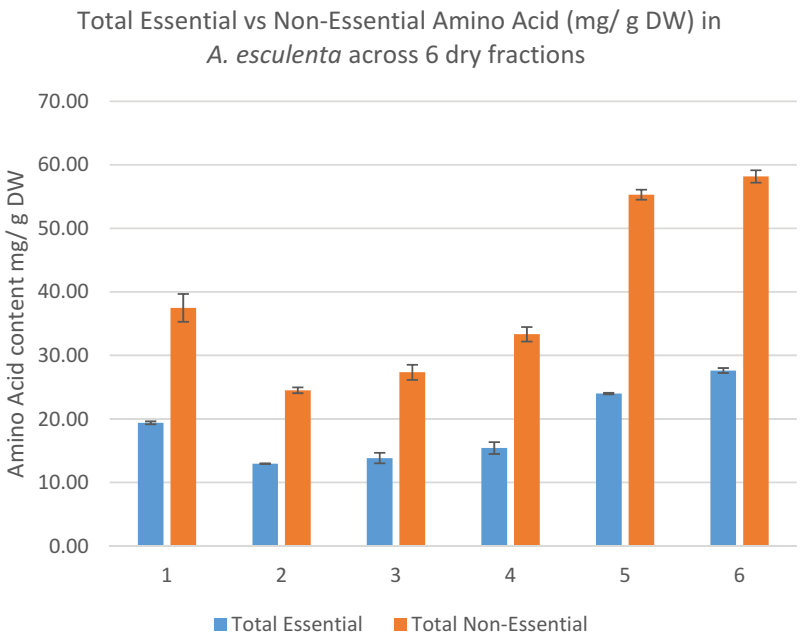

Figure 4. Total Essential vs Non-Essential Amino Acid (mg/ g DW) content in A. esculenta across six dry fractions (Where F1 = >710 µm, F2 = 500–710 µm, F3 = 250–500 µm, F4 = 100–250 µm, F5 = 50–100 µm, F6 = <50 µm).

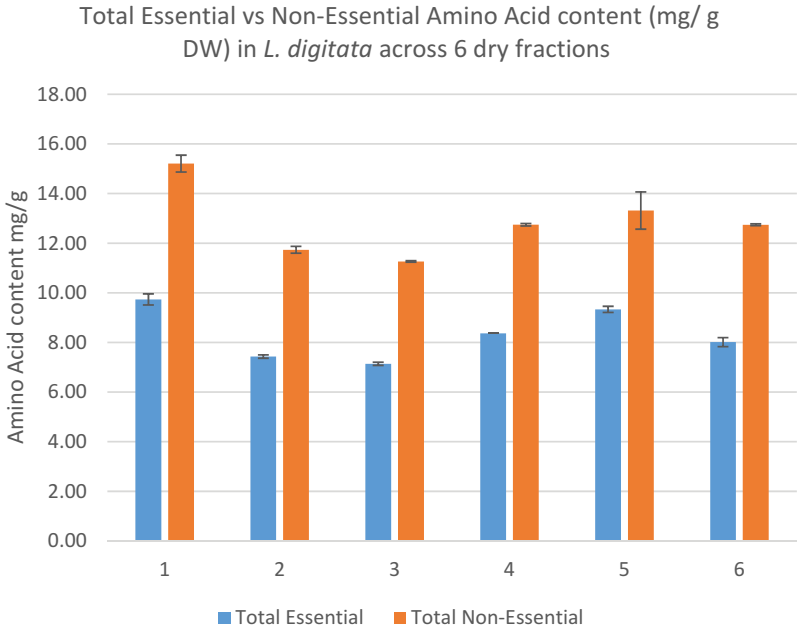

Figure 5. Total Essential vs Non-Essential Amino Acid (mg/ g DW) content in L. digitata across six dry fractions (Where F1 = >710 µm, F2 = 500–710 µm, F3 = 250–500 µm, F4 = 100–250 µm, F5 = 50–100 µm, F6 = <50 µm).

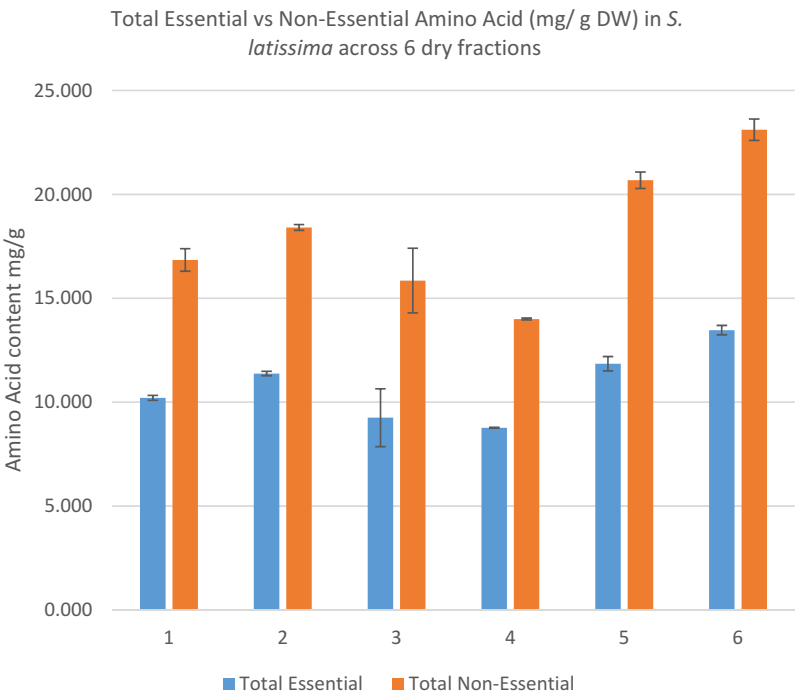

Figure 6. Total Essential vs Non-Essential Amino Acid (mg/ g DW) content in S. latissima across six dry fractions. (Where F1 = >710 µm, F2 = 500–710 µm, F3 = 250–500 µm, F4 = 100–250 µm, F5 = 50–100 µm, F6 = <50 µm).

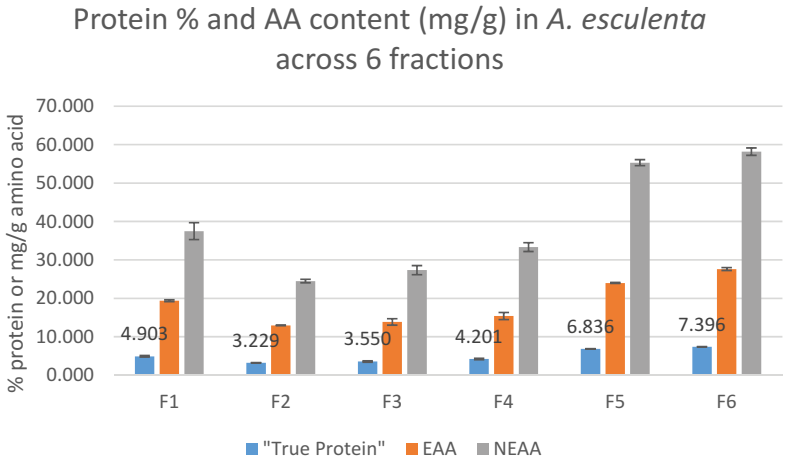

Figure 7. Protein % and AA content (mg/g) in *A. esculenta* across 6 fractions (Where F1 = >710 µm, F2 = 500–710 µm, F3 = 250–500 µm, F4 = 100–250 µm, F5 = 50–100 µm, F6 = <50 µm).

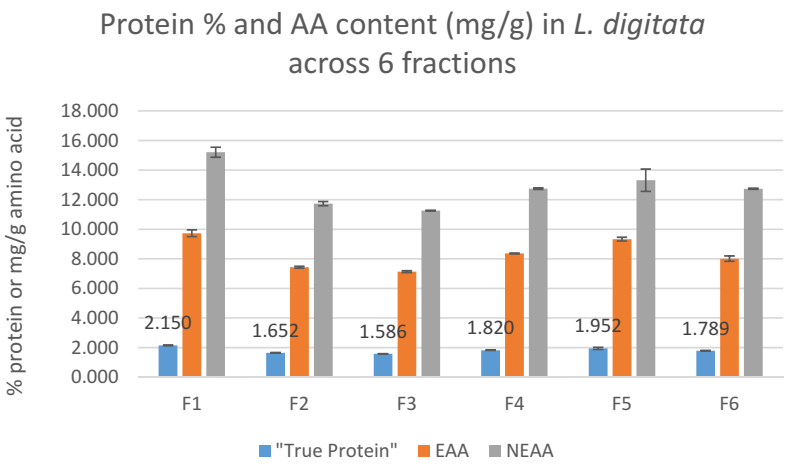

Figure 8. Protein % and AA content (mg/g) in *L. digitata* across 6 fractions (Where F1 = >710 µm, F2 = 500–710 µm, F3 = 250–500 µm, F4 = 100–250 µm, F5 = 50–100 µm, F6 = <50 µm).

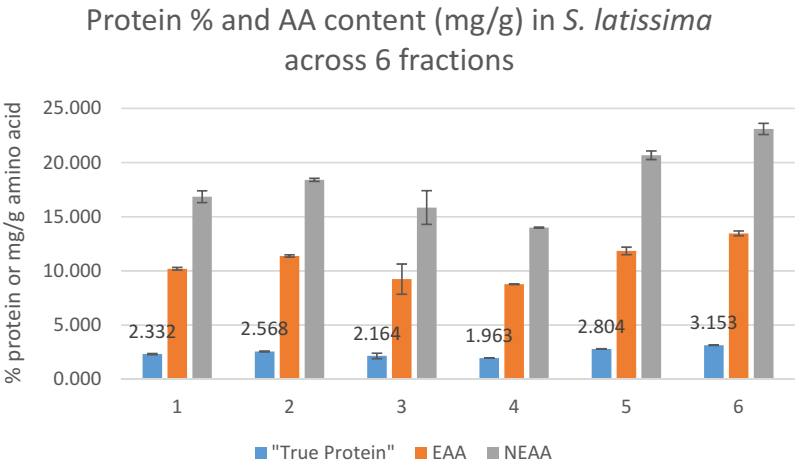

Figure 9. Protein % and AA content (mg/g) in *S. latissima* across 6 fractions (Where F1 = >710 µm, F2 = 500–710 µm, F3 = 250–500 µm, F4 = 100–250 µm, F5 = 50–100 µm, F6 = <50 µm).

Table 6. Levels of significance of protein content (true protein, as determined by total amino acid content) between groups of fractions within three seaweed species (* indicates a significant value).

Fraction interaction	*A. esculenta*	*L. digitata*	*S. latissima*
2–1	$p < .01$*	$p < .01$*	$p < .01$*
3–1	$p < .01$*	$p < .01$*	$p < .01$*
4–1	$p > .05$	$p > .05$	$p < .01$*
5–1	$p < .01$*	$p < .01$*	$p > .05$
6–1	$p < .01$*	$p < .01$*	$p < .01$*
3–2	$p > .05$	$p > .05$	$p > .05$
4–2	$p < .05$*	$p < .05$*	$p > .05$
5–2	$p < .01$*	$p < .01$*	$p < .01$*
6–2	$p < .01$*	$p < .01$*	$p > .05$
4–3	$p > .05$	$p > .05$	$p < .01$*
5–3	$p < .01$*	$p < .01$*	$p < .01$*
6–3	$p < .01$*	$p < .01$*	$p > .05$
5–4	$p < .01$*	$p < .01$*	$p > .05$
6–4	$p < .01$*	$p < .01$*	$p > .05$
6–5	$p > .05$	$p > .05$	$p < .01$*

nutritional composition, fractionation is a cheap and straight-forward process to implement. Without further chemical, enzymatic or mechanical processes, complete separation of protein from polysaccharides is not feasible. Fractionation allows the seaweed producer to make seaweed powders of varying nutritional compositions, while keeping all of his biomass and not producing any waste in the process.

Conclusion

Fractionation is a non-thermal, nondestructive and low-energy consuming process that can be used to separate seaweed powder into fractions of different particle sizes. This allows the seaweed producer to easily generate new products and segment their product line further. As previously mentioned, different fractions of seaweed powder have differing protein and amino acid contents. The seaweed producer may wish to target different markets with their products such as a high-protein seaweed powder for the nutritional supplement market and a lower-protein powder for the food ingredients market. It can also be suggested that further processing such as the extraction of functional ingredients like alginates can be obtained from a certain fraction, leaving the seaweed producer with usable product from other fractions and does not need to direct all of their stock toward functional ingredient extraction. It would first need to be determined which fractions contain the highest yield of these polysaccharides, which requires further research. This study shows that fractionation has a significant impact on the protein content and amino acid profiles of three seaweed species. Further analysis of the distribution of other nutritional compounds like fatty acids or functional polysaccharides across fractionated seaweed should be carried out in order to achieve a bioeconomy approach in the potential application of these different seaweed powders.

Acknowledgments

The authors would like to thank Dúlra for their support and contribution of seaweed for research. M. Pojić would like to acknowledge the financial support of the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract No. 451-03-68/2022-14/200222).

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This research was financially supported by Teagasc Walsh Scholarship and the BiOrbic SFI Bioeconomy Research Centre, which is funded by Ireland's European Structural and Investment Programmes, Science Foundation Ireland (16/RC/3889) and the European Regional Development Fund.

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